



Selection of Potential Plants for Saponin Extract Using Supercritical-CO₂ Extraction against Golden Apple Snails (*Pomacea canaliculata*) for Paddy Cultivation

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Abstract

Golden apple snail, (*Pomacea canaliculata*) has become a major constraint to the profitability of rice (*Oryza sativa* L.) farming by damaging rice seedlings during paddy growth. Chemical compounds such as saponin, alkaloid and flavanoid have been found in pickerel weed (*Monochoria vaginalis*), tea leaves, neem and their usage caused the mortality of *Pomacea canaliculata*. The use of *Furcraea selloa* var. *marginata* at paddy field is effective, practical, green to environment and low cost for decreasing golden apple snails. In this research, three plants have been selected which are *Furcraea selloa* var. *marginata*, Spent Tea Leaves (STL), and *Monochoria vaginalis*. The result of *Monochoria vaginalis* exhibited highest yield of 0.82 wt% followed by STL of 0.75 wt% and *Furcraea selloa* var. *marginata* of 0.52 wt% respectively at the best parameter ranges using Supercritical-CO₂ extraction. Effectiveness tests of biopesticides were carried out on golden apple snails at lab condition by converting the plant extracts as the additives to biopesticide and were sprayed onto the snails. Application of biopesticide based plant extract on golden apple snails exhibited that *Furcraea selloa* var. *marginata* and STL extracts showed higher mortality rate up to 90% compared to *Monochoria vaginalis* based bio-pesticide.

Keywords

Saponin, *Furcraea selloa* var. *marginata*, Compost tea, *Monochoria vaginalis*, Golden apple snail

Introduction

Golden apple snails (*Pomacea canaliculata*) were introduced in Asian countries about 30-years-ago for human consumption. But, it has been reported that the golden apple snail is one of the 100 destructive pests in the world by Ricardo San Martin, et al. [1]. The introduction of the water snails to Asia became a major constraint to the paddy growth activity and yield in paddy cultivation [2]. These snails' mollusks devour young rice seedlings, causing damage to transplant and direct-seed rice [3]. Infestation of golden apple snail on paddy was within 10 to 30 days of paddy age and normally the farmer will make the control for the pest before seedling the seeds into paddy field [4].

Development of food production to meet growing world population and global food demands have led to an increase of chemical inputs in agriculture [2]. For example, to control these snails, synthetic molluscicides chemicals are mainly used such as metaldehyde, copper

sulfate, niclosamide. It is also extremely toxic to the environment and non-targeted organisms [1,2]. Ricardo San Martin, et al. reported that molluscicide based on quinoa (*Chenopodium quinoa*) saponins had successfully eliminated 100% of golden apple snails under laboratory conditions at 24 hours with no toxicity effect on fish, such as goldfish or tilapia [1].

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Saponins are natural tensoactives present in many plants. There are two main types of saponins which are triterpenic and steroidal saponins [1,5,6]. The use of saponin plants to control aquatic snails that transmit schistosomiasis and fascioliasis is well known and has been investigated for many years [1]. Chemical compounds like saponin, alkaloid and flavanoid have been found in *Monochoria vaginalis*, tea leaves, neem and their usage caused the mortality of *Pomacea canaliculata* [7,8]. Besides, *Monochoria vaginalis* is one of the weeds that grows in paddy field and also distract the paddy growth as it will cover the surface of water thus transferring less oxygen to paddy [9]. According to Joanne L, et al. there are four steroidal saponins that were isolated from the leaves of *Furcraea selloa* var. *marginata* which are one furostanol saponin, fucraea furostatin and three known spirostanol saponins, furostatin, yuccaloeside C and cantalasaponin-1 [10]. Besides, the use of *Furcraea selloa* var. *marginata* powder at paddy field is effective, practical, green to the environment and is cost effective to reduce the golden apple snails compared to chemical pesticide [11]. Triterpenoid saponins are important bioactive compound in tea and over 60 kinds of triterpenoid saponins have been isolated and characterized in the leaves, seeds, roots and flowers of oolong tea (*Camellia sinensis*) [12]. In addition, saponin is usually produced by plants as pathogen agents and herbivores as it has immune stimulatory properties, anticancer, antimicrobial, anti-fungal, anti-inflammatory and antiviral activities [13].

Moreover, the Supercritical Fluid Extraction (SFE) is one of the most useful and effective methods for extracting active components from plants because of many advantages of carbon dioxide which are non-toxic, non-flammable and cheap [14].

Thus, this preliminary test was carried out to select the potential plants which are *Furcraea selloa* var. *marginata*, STL, and *Monochoria vaginalis* in order to extract saponin compound using Supercritical-CO₂ extraction as an additive in biopesticide. In addition, the mortality test of *Pomacea canaliculata* was also performed.

Materials and Methods

Feedstock

Furcraea selloa var. *marginata* and *Monochoria vaginalis* were collected at Biosecurity Plant Unit of Agriculture Department of Parit Buntar, Perak, Malaysia. The STL were collected from a restaurant at Sri Iskandar, Perak, Malaysia. All the feedstocks were dried a temperature of 60 °C until the moisture content was ± 10%. The feedstocks were then ground using a grinder into selected particle sizes range from 0.15 to 0.35 mm.

Supercritical-CO₂ extraction

Supercritical-CO₂ extractions were performed at dif-

ferent work conditions of pressure, temperature and CO₂ flow rate. The operating procedure was carried out by filling the extraction cell with 5 ± 0.05 g of feedstock. A thermoresistance around the extractor was maintained at the desired temperature. The CO₂ gas was compressed through a high-pressured pump and preheated before flowing through the extraction vessel. The plant extract was collected in the tube covered with aluminum foil to avoid light as the properties of plant extract is light sensitive. The yields of plant extracts were calculated using Equation (1) [14,15]:

$$\text{Yield, } Y \text{ (wt\%)} = \frac{\text{mass of plant extract}}{\text{mass of feedstock}} \times 100\% \quad (1)$$

Gas Chromatography-Mass Spectrometry (GC-MS) analysis

The plant extracts were dissolved in ethyl acetate and were analyzed by using Agilent Technologies 7890 A GC combined with a 5975 C triple quadrupole MS. The GC system was equipped with a purged tee installed after the analytical column (30 m length × 0.25 mm ID × 0.25 μm DB-5MS) in order to perform a back flush of low volatile material, reducing contamination of the analytical column. The oven temperature program was adjusted to 100 °C (3 min) - 10 °C/min to 320 °C (25 min). A time-programmed 10 μL injections was performed in solvent vent mode using a Gerstel PTV. The LVI-PTV conditions were as follows: Injection speed was 0.2 μL/s and vent flow was 120 ml/min [16]. The plant extracts were compared with the saponin standards to determine the presence of saponins in the samples.

Efficacy test of biopesticides on the *Pomacea canaliculata*

The golden apple snails were collected from drainage system of paddy field which is located at Parit Buntar, Perak, Malaysia and the snails were kept in containers with food. According to Alexander M. Stuart, the juvenile snail is in the range of 10 to 15 mm and the adult snail is in the range of 20 to 25 mm. For this preliminary test, ten active adult golden apple snails were selected for each test. The 5 cm depth of soil was filled in the containers (20 cm × 20 cm) by adapting paddy cultivation environment [2]. *Furcraea selloa* var. *marginata*, STL and *Monochoria vaginalis* based biopesticides were prepared by diluting the plant extract with purified water with ratio of 1:1000 by volume with 5% of ethyl acetate solution. The conversion of plant extract as the additive in biopesticide is referred to the procedure described by Yuningsih, et al. and Ravindra C Joshi, et al. [3,17]. Four conditions were carried out in which three containers were sprayed with three different types of biopesticides and one container was sprayed with purified water as the control until all the golden apple snails immersed in the

Table 1: Best range of parameters for Supercritical-CO₂ extraction of saponin compound.

1. Particle size, mm		
Feedstock	Particle size range, mm	Yield (weight%)
<i>Monochoria vaginalis</i>	0.15-0.35	0.82
Spent tea leaves	0.15-0.35	0.75
<i>Furcraea selloa</i> var. <i>marginata</i>	0.15-0.35	0.52
2. Pressure, MPa		
Feedstock	Pressure range, MPa	Yield (weight%)
<i>Monochoria vaginalis</i>	20-25	0.82
Spent tea leaves	20-25	0.75
<i>Furcraea selloa</i> var. <i>marginata</i>	20-25	0.52
3. Temperature, °C		
Feedstock	Temperature range, °C	Yield (weight%)
<i>Monochoria vaginalis</i>	40-50	0.82
Spent tea leaves	40-50	0.75
<i>Furcraea selloa</i> var. <i>marginata</i>	40-50	0.43
4. CO₂ Flowrate, ml/min		
Feedstock	CO₂ flow rate range, ml/min	Yield (weight%)
<i>Monochoria vaginalis</i>	5-8	0.82
Spent tea leaves	5-8	0.75
<i>Furcraea selloa</i> var. <i>marginata</i>	5-8	0.52

solutions of about 2 inches water level; which adopted the standard technique following the standard pest control in paddy cultivation [4]. The purified water was used as the control as there was a possibility that the water could also cause the mortality of the snail when the environment is different than paddy field. Thus, to avoid the error in this study, the water was used as control as the biopesticides were produced by diluting the plant extracts in purified water. The test was also replicated with 3 replications at each condition to increase the accuracy. The mortality rate was recorded for 12, 24, 36 and 48 hours after bio-pesticides been applied.

Results and Discussion

Yield of essential oil

The supercritical-CO₂ extractions were carried out to determine the best range of particle size, pressure, temperature, and CO₂ flow rate to have higher yields which adopted the parameter range of Supercritical-CO₂ extraction described by Kamarulzaman PSD, et al. as the guideline [18]. This study conducted extraction process using several range of particle sizes which are 0.15 to 0.19 mm, 0.2 to 0.29 mm and 0.3 to 0.35 mm; several range of pressures which are 20 to 25 MPA and 26 MPA to 30 MPA; several range of temperatures which are 40 to 49 °C and 50 to 60 °C and several range of CO₂ flow rates which are 3 to 4 ml/min and 5 to ml/min as screening data for further Research Surface Methodology (RSM) application in order to find the optimum parameters for these extractions. Table 1 described the best range of the parameters for the Supercritical-CO₂ extraction of plant extracts using selected plant which showed the highest yield compared to other range. However, the range of

particle size and CO₂ flow rate showed no difference in yield compared to other parameters.

Table 1 showed *Monochoria vaginalis* extract produced highest yield of plant extracts of 0.82 wt% followed by STL of 0.75 wt% and *Furcraea selloa* var. *marginata* of 0.52 wt% respectively using the best parameters range of Supercritical-CO₂ extraction. The purpose of RSM measurement is to evaluate the effect of several factors and their interaction on one or more response variables [14]. By using this method, it will reduce the experimental runs and provides statistically acceptable optimum parameters to produce higher yield for these extractions. In addition, the application of supercritical-CO₂ extraction technology to the processing of polar solutes such as saponin compound is limited by low solvent power of the extracts for these solutes [19] which can be improved by using water or ethanol as cosolvent to increase supercritical-CO₂ polarity [13]. Besides, Mamata also said that for the extraction of a certain class of products, a cosolvent or an entrainer is often injected into supercritical-CO₂ to increase its polarity and hence it's solvent power. Ethanol, ethyl acetate and possibly water are the best natural entrainers for food-grade products [20], are less harmful to the environment which are also better solvents to produce biopesticide. According to Raphaela, et al., the extracts from the Supercritical-CO₂ extraction with ethanol as cosolvent with (70:30, w/w) showed the greatest ability to reduce the surface tension of water and was the best for extraction of less polar saponins in the extract [13]. This proven that a careful choice of cosolvent could be used to separate the components, not just on the basis of polarity, but also on the basis of functional groups and the ability to have specific interactions [20]. Thus, this re-

Table 2: Similar chemical compound in the plant extracts.

Chemical compound	Plant extract	Retention time (min)	Area of peak (%)	Application
D-Limonene	Saponin standard	-	-	Antimicrobial, antifungal, antioxidant, as cleaning liquids, cosmetics and flavorings [33-35].
	<i>Furcraea selloa</i> var. <i>marginata</i>	3.486	1.09	
	Spent tea leaves	3.486	0.32	
	<i>Monochoria vaginalis</i>	3.486	2.20	
Cyclohexasiloxane, dodecamethyl	Saponin standard	7.412	7.51	Cleaning agents, cosmetics, textile applications, antifungal and as biological resistance to termites [21,22].
	<i>Furcraea selloa</i> var. <i>marginata</i>	7.404	5.57	
	Spent tea leaves	7.412	1.64	
	<i>Monochoria vaginalis</i>	7.412	6.07	
Cycloheptasiloxane, tetradecamethyl	Saponin standard	9.763	11.87	Cleaning agents, cosmetics, textile applications, antifungal and as biological resistance to termites
	<i>Furcraea selloa</i> var. <i>marginata</i>	9.756	7.22	
	Spent tea leaves	9.763	2.59	
	<i>Monochoria vaginalis</i>	9.763	8.48	
Cyclooctasiloxane, hexadecamethyl	Saponin standard	11.790	8.64	Cleaning agents, cosmetics, textile applications, antifungal and as biological resistance to termites
	<i>Furcraea selloa</i> var. <i>marginata</i>	11.790	5.64	
	Spent tea leaves	11.790	1.88	
	<i>Monochoria vaginalis</i>	11.790	5.82	
Cyclononasiloxane, octadecamethyl	Saponin standard	13.508	6.73	Cleaning agents, cosmetics, textile applications, antifungal and as biological resistance to termites
	<i>Furcraea selloa</i> var. <i>marginata</i>	13.508	4.48	
	Spent tea leaves	13.508	1.46	
	<i>Monochoria vaginalis</i>	13.508	4.87	
Tetrasiloxane	Saponin standard	18.813	4.86	Cleaning agents, cosmetics, textile applications, antifungal and as biological resistance to termites
	<i>Furcraea selloa</i> var. <i>marginata</i>	18.813	4.99	
	Spent tea leaves	18.805	1.68	
	<i>Monochoria vaginalis</i>	18.805	4.41	
Heptasiloxane	Saponin standard	20.923	5.09	Cleaning agents, cosmetics, textile applications, antifungal and as biological resistance to termites
	<i>Furcraea selloa</i> var. <i>marginata</i>	20.923	5.62	
	Spent tea leaves	20.923	1.95	
	<i>Monochoria vaginalis</i>	20.923	5.23	

search will study in future by selecting the best cosolvent to increase the yield of the plant extracts.

Chemical compound in plant extracts

The study was carried out using saponin standard in order to determine the saponin compound in the plant extracts. The standard was compared to each *Furcraea selloa* var. *marginata* extract, STL extract and *Monochoria vaginalis* extract which were dissolved in ethyl acetate solution. The GC-MS analysis is unable to detect saponin compound directly due to the high polarity of saponin. The analysis showed that most of the peaks of the sample had similar peaks with saponin standard as described in Table 2 and Figure 1. This study will analyze the sample

further in future to determine the qualitative and quantitative analysis of saponin in the plant extracts using High Pressure Liquid Chromatography (HPLC) by following the procedure described by Fang Liu, et al. [6].

Table 2 showed the chemical compounds that have same retention time with the saponin standard such as cyclooctasiloxane and hexadecamethyl at the retention time of 11.79 minutes, cyclononasiloxane and octadecamethyl at the retention time of 13.508 minutes and heptasiloxane at the retention time of 20.923 minutes. All the compounds have been used in pesticide and insecticide application [21,22]. Moreover, there are also other compounds in each plant extracts that can be toxic to the

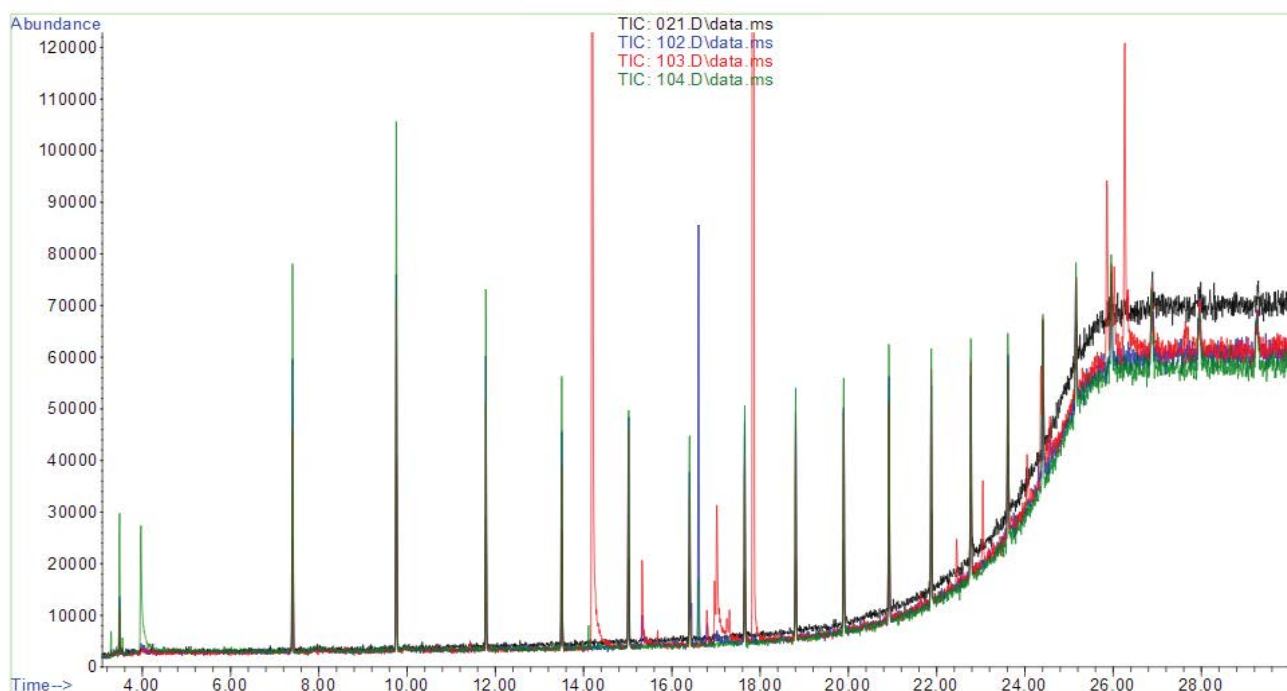


Figure 1: All peak of plant extracts and saponin standard.

*T1C: 021 D\data = Saponin standard; T1C: 102 D\data = *Furcraea selloa* var. *marginata* extract;

T1C: 103 D\data = STL extract and; T1C: 104 D\data = *Monochoria vaginalis* extract.

insect, pest and bacteria. For examples, Imidazole which was found in *Furcraea selloa* var. *marginata* extracted at the retention time of 16.447 minutes with 0.99% of peak area was used not only in chemistry and pharmacology [23], but Imidazole was also used for derivatives process of biological activities such as antibacterial, anticancer, antitubercular, antifungal, analgesic and anti-HIV activities [24]. Benzofuranone was also found in the *Furcraea selloa* var. *marginata* extracted at a 29.264 of retention time with 2.15% of peak area. The compound is well known as antioxidant agent [25], to prevent the oxidative degradation of variety materials and improve the stability of polymer in high temperature process [26]. The Benzofuranone also was used in medicine as antinociceptive agents which are the action of blocking the pain detection by sensory neurons [27]. Phytol at retention time of 16.801 minutes with 0.43% of peak area and Beta-amyrin at retention time of 26.265 minutes with 4.94% of peak area which were found in STL extracted was used as antimycobacterial, antifungal and anti-inflammatory [28,29]. Besides, Citronellol was found in the *Monochoria vaginalis* extracted at a retention time of 14.118 minutes with 0.49% of peak area which was used as active compound in repellent for mice and mosquitoes [30,31]. The compound also was reported having high rate and lost lasting repellency against parasites [32]. Based on the application of the chemical compounds in plant extracts, it is proven that they have high potential to be the effec-

tive biopesticides for pests and bacteria.

Efficacy test of biopesticides against *Pomacea canaliculata* population

Based on three replications of efficacy test of biopesticides against golden apple snail population, biopesticides based plant extracts were successfully increased the mortality rate up to 90% within 48 hours observation as per described in Table 3 and Figure 2. Ravindra C Joshi, et al. reported that the most resistant snails corresponded to quinoa saponin have a size of 15-20 mm [3]. This study used the snails with the size range of 20 to 25 mm and the result showed that the plant extracts were successful in causing the mortality of the snails. Thus, *Furcraea selloa* var. *marginata* and STL extracts might have higher concentration of saponin compared to quinoa saponin. Although *Furcraea selloa* var. *marginata* and STL extracts produced lower yields compared to the *Monochoria vaginalis* extract from Supercritical-CO₂ extraction, they managed to have a higher mortality rate of *Pomacea canaliculata* population, which are 90% mortality rate compared to *Monochoria vaginalis* extract with 70% mortality rate after 48 hours application of biopesticides based on the plant extracts. There is a possibility that *Furcraea selloa* var. *marginata* and STL extracts have higher saponin compound than *Monochoria vaginalis* extract. However, based on GC-MS analysis of plant extracts, there are also other compounds such as essential

Table 3: Mortality rate of *Pomacea canaliculata* (%).

Biopesticide based	Mortality rate of <i>Pomacea canaliculata</i> (%)															
	12 hours				24 hours				36 hours				48 hours			
	S1	S2	S3	Mean	S1	S2	S3	Mean	S1	S2	S3	Mean	S1	S2	S3	Mean
<i>Furcraea selloa</i> var. <i>marginata</i> extract	50	30	40	40	60	60	60	60	70	70	100	80	70	100	100	90
Spent tea leaves extract	20	30	30	30	30	60	50	50	50	100	80	80	60	100	100	90
<i>Monochoria vaginalis</i> extract	20	0	0	10	40	30	30	30	50	60	60	60	50	60	70	60
Purified water (control)	0	0	0	0	0	0	0	0	0	0	0	0	10	0	0	10

S1 = Replication 1, S2 = Replication 2 and S3 = Replication 3.

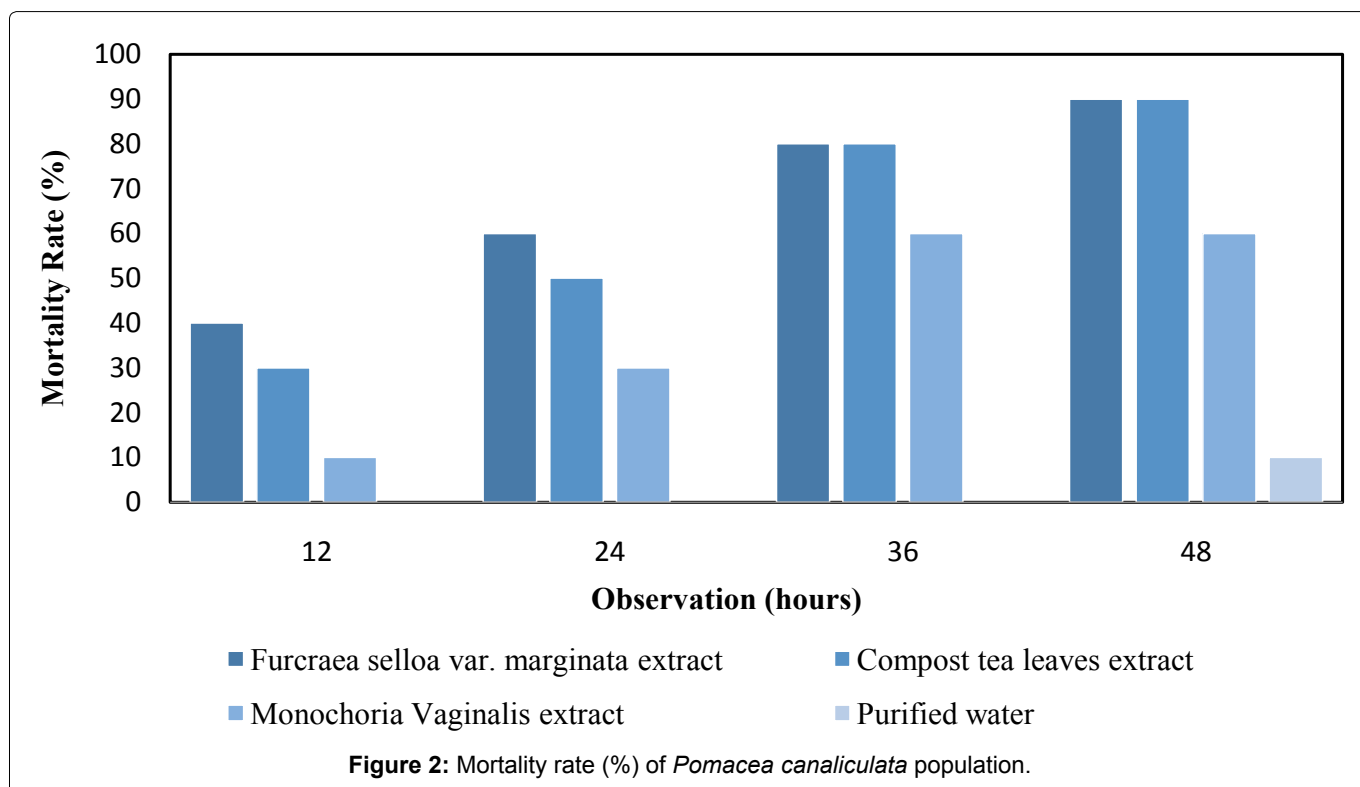


Figure 2: Mortality rate (%) of *Pomacea canaliculata* population.

oil which can also cause the mortality of the golden apple snail. However, the analysis failed to detect the saponin compound directly it only showed that most of the peaks in the plant extracts have similar peaks with saponin standard. Therefore, the actual concentration of saponin in the plant extracts using High Pressure Liquid Chromatography (HPLC) will be determined in future. In addition, ethyl acetate solution also influences the increase of the mortality rate of the golden apple snails. Based on the positive outcome, *Furcraea selloa* var. *marginata* and STL have been chosen for further study in terms of the optimization process of extraction and formulation of biopesticide based on combination of plant extracts using Supercritical-CO₂ extraction.

Conclusion

This preliminary test was successfully proven that *Furcraea selloa* var. *marginata* and STL extracts have higher potential to be the additives in biopesticide to in-

crease the mortality rate of *Pomacea canaliculata* population in paddy cultivation. Therefore, the study will continue to develop effective biopesticide based saponin by using the combination of both plant extracts as the active compound in the biopesticide. The determination for the optimum parameters using RSM, quantification of saponin using HPLC analysis and the toxicology test of paddy will be carried out further in future.

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